Rapid establishment of the European Bank for induced Pluripotent Stem Cells (EBiSC)

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Rapid establishment of the European Bank for induced Pluripotent Stem Cells (EBiSC) - the *Hot Start* experience

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[1] As of 1 December 2015, Roslin Cells Ltd.'s role in EBiSC was assumed by Roslin Cells Sciences Ltd. which is now a wholly own subsidiary of Censo Biotechnologies Ltd. (whose Head Office is Wallace Building, Roslin Biocentre, EH25 9PP).

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ABSTRACT

A fast track "*Hot Start*" process was implemented to launch the *European Bank for Induced Pluripotent Stem Cells* (EBiSC) to provide early release of a range of established control and disease linked human induced pluripotent stem cell (hiPSC) lines. Established practice amongst consortium members was surveyed to arrive at harmonised and publically accessible Standard Operations Procedures (SOPs) for tissue procurement, bio-sample tracking, iPSC expansion, cryopreservation, qualification and distribution to the research community. These were implemented to create a quality managed foundational collection of lines and associated data made available for distribution. Here we report on the successful outcome of this experience and work flow for banking and facilitating access to an otherwise disparate European resource, with lessons to benefit the international research community.

eTOC: The report focuses on the EBiSC experience of rapidly establishing an operational capacity to procure, bank and distribute a foundational collection of established hiPSC lines. It validates the feasibility and defines the challenges of harnessing and integrating the capability and productivity of centres across Europe using commonly available resources currently in the field.

1. Introduction

Since their introduction in 2007 (Takahashi et al., 2007) hiPSC have been rapidly and broadly incorporated into research to understand their potential for disease. This has substantiated interest to incorporate this resource into drug discovery pipelines, prospective patient stratification, recruitment for clinical trials and post-clinical drug assessment of safety issues following rare event reporting. However, the evolution of these applications depends on facilitated and unfettered access to a standardised and well characterised hiPSC resource to help avoid dissemination of unauthenticated or substandard cell lines to the research community. Rising demand by academia and industry has instigated a number of large scale public and privately funded disease and/or population oriented hiPSC banking initiatives in the US, Japan and UK (McKernan and Watt, 2013). These will foreseeably improve the consistency with which new cell lines will be developed but not necessarily the standardised and scalable distribution of pre-established or new lines to the wider hiPSC research community.

At present in Europe, a human pluripotent stem cell registry (www.hPSCreg) offers the research community, legislators, regulators and the general public a measured overview of the current status of human pluripotent stem cell research though the provision of information on established cell lines derived from embryos or induced from adult cell sources. At time of writing over 452 hiPSC lines are on the registry, sponsored by EU, nation specific and charitable funding sources. However, this resource does not facilitate standardise access to these resources which still require user interface with institutional representatives in possession of any given cell line. Recognising a need for a harmonised framework of best practice, the Innovative Medicines Initiative Joint Undertaking (IMI JU), a pan-European public private partnership between the European Commission (EC) and European Federation of Pharmaceutical Industries and Associations (EFPIA; www.efpia.eu), issued a call for proposals which in January 2014 culminated in the launch of the European Induced Pluripotent Stem Cell Bank (EBiSC). EBiSC's mission has been to: i) identify key cohorts of patients useful for research purposes within the wider scientific community, ii) create a large single European hiPSC repository through the integration of existing infrastructure, such as for example hPSCreg, and iii) generate a centre of scientific excellence for standardisation and optimisation of cryopreservation, retrieval and differentiation methods for hiPSC lines. Here we report on the experience of the first stage of this initiative, a fast track "*Hot Start*" process whose goal was to rapidly establish operational capacity and a distributable foundational collection of established hiPSC lines.

2. Results

The *Hot Start* experience and outcomes were embodied in established and conceived infrastructure provided by its membership. This was both as originally proposed and subsequently informed and amended in the course of operational experience as follows.

2.1. Infrastructure set up

2.1.1. EBiSC Consortium membership and organisation

The founding EBiSC membership consisted of 6 EFPIA partners defining industrial demand for a standardised hiPSC resource, and 23 non-EFPIA partners. The latter consisted of 6 Small Medium-sized Enterprises (SME), 15 academic/research institutions and two governmental agencies (Supplementary Fig. 1). Contribution of members was organised in a 7 work-package (wp) programme addressing management (wp1; governance, policy, hiPSC market assessment, communication and dissemination), creation of the bank (wp2; procurement, operation and sustainability), process improvement (wp3; cryopreservation and automation), public engagement (wp4; ethics, training), quality control and characterisation (wp5), end use (wp6; storage, distribution and product validation), and data management (wp7; information management system development and long-term operation). At the outset of the programme a consortium participant agreement stipulated that the hiPSC lines deposited in EBiSC could be used for research by both for profit and not for profit organisations and the ongoing rights of access of all participants to the new knowledge created as part of the *Hot Start* process.

2.1.2. Workflow design and outputs

The focus of the *Hot Start* process was to establish a workflow between hiPSC supplying centres, a Central Bank or Facility for quality control, and ancillary sites responsible for storage, management and distribution of information and cells to users (Fig. 1). This effort culminated in the establishment of the *Hot Start* collection comprised of established hiPSC lines and related data and clinical links provided by 7 research organisations across 5 European member states (UK, Germany, Denmark, Netherlands, and Spain). It also resulted in the establishment of a bespoke information management system (IMS) supporting the storage, organisation and presentation of EBiSC data to accommodate diverse user interests: Cell line depositors, communicating resource details; EBiSC staff using the IMS to track internal operations; bioinformaticians requiring programmatic access to well-structured data; and prospective cell line users procuring lines in accordance with selection criteria such as donor disease representation and methodology used to create the resource.

2.1.3. Definition of minimum essential information

The de facto variability across supplying centres in the provenance of hiPSC lines (i.e. terms of consent, information available on donors and lines and methods applied to generate the lines), required definition of minimum essential attributes to accept established lines for deposition in the bank and consensus operational standards and procedures.

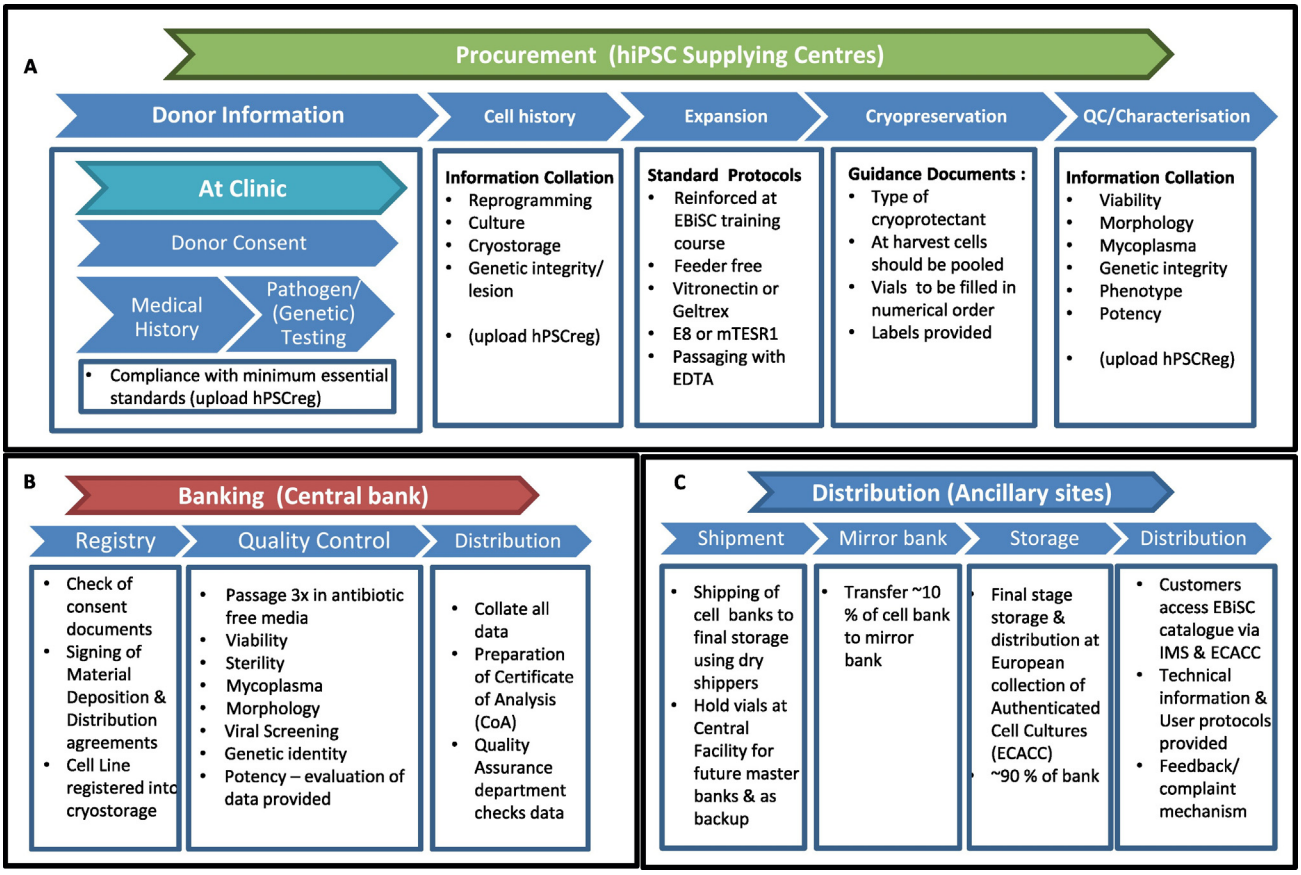

Fig. 1. EBiSC *Hot Start* harmonised banking of established hiPSC lines. A: Consensus standardised protocols were implemented to collect and upload pre-existing donor and cell line information to HPSCreg and expand, cryopreserve and ship hiPSC lines to Central Bank. B: At the Central Facility cell lines were registered and evaluated as part of the standardised quality control procedure. C: Approved hiPSC line batches were shipped for final storage and worldwide distribution by e-commerce.

As regards donor consent, minimum criteria for hiPSC line deposition included:

i) That the donation was voluntary, without financial or other benefit;
ii) Donor de-identification and traceability;
iii) Definition of scope and/or restrictions on permitted use of donated material;
iv) Potential for access via providing centre to genetic and clinical information made available with the primary cell donation;
v) No expectation of feedback regarding incidental findings, data or information arising from research use of cell lines.

Given that several iPSC lines identified of interest for initial use were originally not consented explicitly or had unclear conditions of consenting for use by for profit organisations, the decision was made to not use this as an exclusion criterion, as necessary reconsenting donors. How readily hiPSC supplying centres provided minimal essential information varied in accordance with the strength of pre-existing relationships with clinical centres providing access to donors.

2.1.4. Standardisation of methods and labelling

Standard documents drafted and implemented as part of the establishment of the *Hot Start* collection included: i) forms and protocols for the collection of historical information on donor and cell line history; and ii) procedures for hiPSC culturing and banking. The former included the essential requirement to define clear cell line ownership for subsequent use of an EBiSC standardised Material Deposition and User Access Agreements. An EBiSC template for Patient Information and Consent (PIC), was useful in the event the terms of the original PIC used by the derivation centre failed to comply with minimum essential requirements and it was possible to re-consent the donor. Standard culture and banking procedures included protocols for feeder-free hiPSC growth, cryopreservation and storage, batch specific labelling, testing, release and shipment at the hiPSC supplying research centres, Central and Mirror Banks and distribution centres (See Experimental Procedures and Supplementary Figs. 2,3).

Labels were provided by the Central Bank and founded on the principles of hiPSC line identification previously established by the European hPSCreg registry (www.hPSCreg.eu; Seltmann et al., 2016). Label design and nomenclature evolved over 24 months from prototypes to enable the incorporation of operational feedback and requirements (Fig. 2). Changes encompassed inclusion/omission of depositor derived identifiers, machine readable identifiers compatible with existing operational systems (eg. Askion automated cryopreservation, Neubauer et al., 2015; European Collection of Authenticated Cell Cultures), unique biosample accession numbers (Gostev et al., 2012), EBiSC logo and batch and vial numbers. Partner performance of consensus protocols was reinforced by semi-annual practical and theoretical training courses. Feedback from the first two training courses confirmed majority satisfaction with course effectiveness and utility (Supplementary Fig. 4).

2.1.5. Data management

Pre-existing or hiPSC supplying centre generated Information available for each hiPSC line were uploaded onto hPSCreg. Three categories of information were collated:

i) Background information on the donor and methods used to create the cell line, including donor age, gender, origin, and disease association, genetic lesion/clinical diagnosis, identity of primary

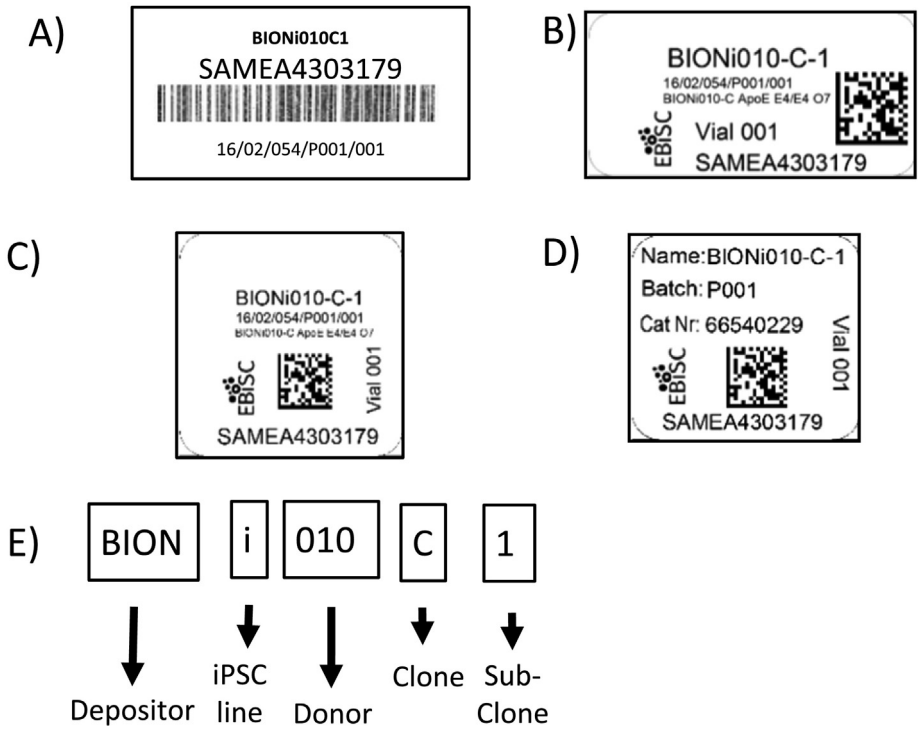

Fig. 2. Standardised nomenclature and labelling. Labelling content and format adhered to principles of hPSC registration established by hPSCreg, and requirements of operator and machine readable identifiers, at the Central and Mirror Banks and distribution centre (Public Health England, ECCAC). This evolved in the course of the first 24 months of the EBiSC *Hot Start* (A–D). Changes included switch from a 1 to 2 dimensional machine readable identifier and incorporation of EBiSC branding (A to B), and inclusion and then omission of supplying centre identifying information (B to D). EBiSC nomenclature (E) identifies depositing supplier (BION, reference to Bioneer A/S), identity as an iPSC line and donor, clone and sub-clone numbers, augmented further by batch, vial and ECCAC catalogue numbers noted separately. Each vial was also given a unique biosamples accession numbers (Gostev et al., 2012; in this example SAMEA4303179).

cells used for cell reprogramming, and reprogramming methodology (genes, vectors, cell culture history);

ii) historical data on hiPSC characterisation, including sterility, viral pathogen screening, pluripotency potential, confirmation of genetic lesion (if known) (Exemplar, Supplementary Fig. 5); and,

iii) quality control data collected in the course of cell expansion and banking for the *Hot Start* process including viability, morphology, mycoplasma, genetic identity (DNA microsatellite PCR) and integrity (karyology) and cell phenotype (minimum expression of 3 pluripotency associated biomarkers).

For each hiPSC line, data was collated to prepare a Certificate of Analysis (CoA) whose accuracy was independently checked by a Quality Assurance team before subsequent distribution of cell lines to the Mirror Bank with a fully automated extended cryopreservation cold chain (Fraunhofer IBMT, http://www.ibmt.fraunhofer.de/en.html), and the distribution centre (ECACC), (Supplementary Fig. 6a,b). As lines were received at the distribution centre they were registered on an Oracle based inventory and stock control system with links into the e-commerce website (https://cells.ebisc.org). On the latter, each hiPSC line is set up with a basic data set providing key information along with downloadable associated documents such as Material Safety Data, technical handling information, Access Use Agreement (AUA), and end user hiPSC culture protocols. The site also provides the facility to download CoA upon entry of the batch number of the cells received by the end user. Upon distribution centre receipt of a signed AUA, hiPSC lines are despatched to end users within ECACC's standard practice of 5 business days worldwide. To date this has included centres in the UK, Germany, Denmark and the USA.

2.2. Operational experience

2.2.1. hIPSC line supply

Of the 7 supplying centres, 4 centres fulfilled or were on course to fulfil all aspects of their commitment to provide established lines as of March 2016. One supplier encountered late-stage reluctance of clinical partners from which cells were donated to accept terms of material deposition and transfer agreements which potentially would make the lines immediately available to the research community prior to depositors' publication of research on the lines being deposited. Two suppliers were unable to locate viable cell line stocks with no viable alternatives available. With the exception of a supplier based in Spain, all centres were able to agree to deposit the lines in a Central Bank and subsequent centralised distribution. The law of Spain requires project specific approval by a national authority and Spanish administrative control over distribution of hiPSC lines to other partners in the EBiSC consortium or third parties. For proof of concept validation of consensus protocols and quality control measures, project specific approval was secured for shipment of the Spanish hiPSC lines to the Central Facility and use therein, demonstrating that even across national sovereign legislation, EBiSC was able to operate to facilitate international cell line transfer.

Initially 7 hiPSC supplying research centres were asked to commit to providing 8–10 hiPSC lines established by them. This yield a final total commitment of 47 hiPSC lines representative of diverse donor health and disease status and clonal variation. Progress against key performance milestones at months 17 and 29 is summarised in Fig. 3. The first hiPSC lines were received by the Central Bank 8 months after the launch of the programme, and distributed directly to a third party user by 12 months. However, it took 17 months until a majority of the centres (4) had deposited both the cell line batches and the required data

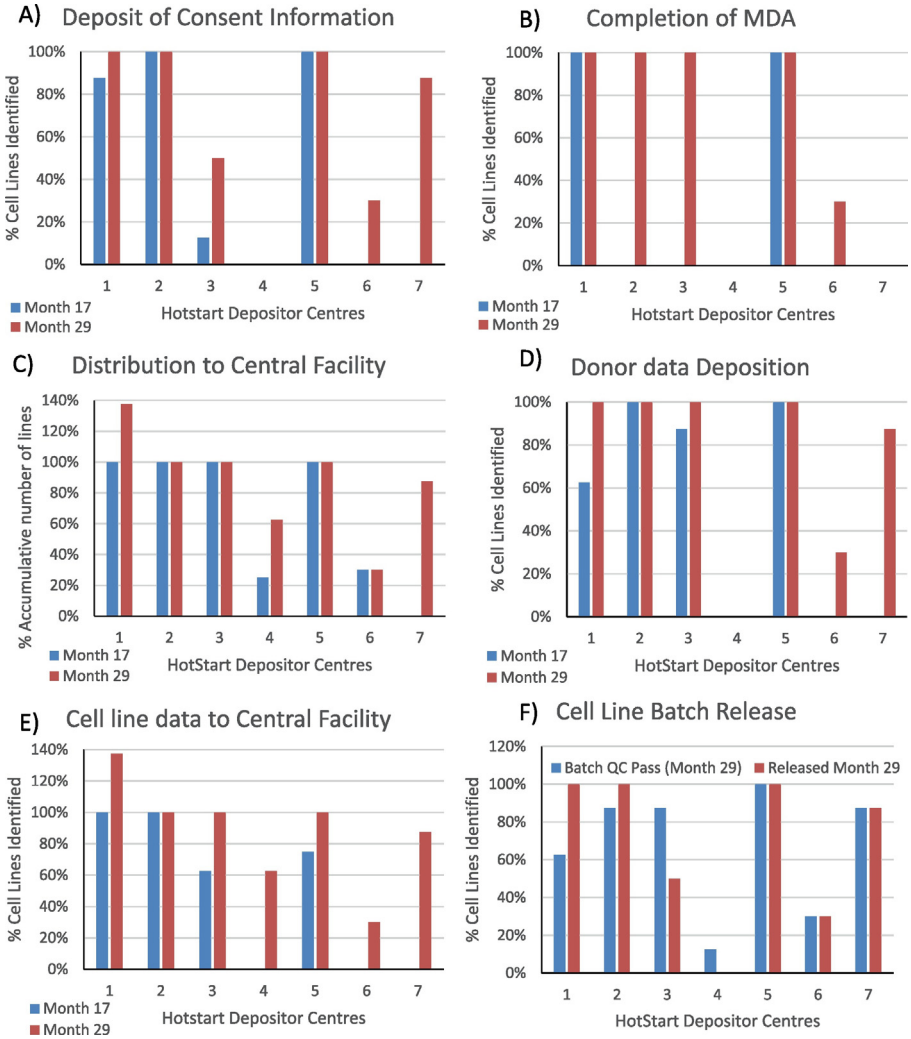

Fig. 3. Key Performance indicators (KPI) of *Hot Start* hiPSC supplying centres. After identification of 8–10 suitable cell lines, centres were required to undergo a number of processes to enable cell line release. After performing expansion and banking, each cell line was distributed to the Central Facility for additional QC, data review and formal release to ECACC. Progress in these KPIs over the project period after Month 17 and 29 (May 2015 & 2016) are shown here as cell line distribution to the Central Bank (A), completion of Material Deposition Agreement (B), distribution to Central Facility (C), anonymised donor data deposition (D), cell line batch data deposition (E) and cell line batch release data to distribution centre (F). *E*-commerce distribution of a specific cell line from the distribution centre required all of these parameters to be fulfilled. All parameters are assessed relative to the number of prospective lines initially identified, increases above 100% are due to depositors being required to repeat cell line batch production and characterisation subsequent to a failure of the first batch.

for a majority of the lines they had committed. After another year (in project month 29) another two reached these endpoints for a fraction of their lines.

Lines released for public distribution represent cell line batches whereby all requirements had been satisfied; consent information had been approved, an MDA had been completed, and the Central Facility has received a batch, and approved characterisation data and the line had passed Quality control at the Central Facility. By the time of the formal launch of the EBiSC catalogue 27 months into the programme on 23rd of March 2016 (https://cells.ebisc.org), 27 of the 47 lines (57%) provided by 5 of the depositors were available by e-commerce. To validate the pluripotent phenotype of ECACC distributed lines a selected line (UKBi005-A, depositor and EBiSC QC data for which is provided in Supplementary Fig. 5 for) was procured from the distributing centre, expanded and submitted to a high content imaging analysis focussing on the pluripotency characteristics of the material provided. Immunofluorescent staining and quantification of pluripotency markers OCT3/4/POU5F1, SOX2, NANOG and LIN28 revealed high amounts of positive cells under consensus culture conditions (91% OCT3/4/POU5F1, 81% SOX2, 73% NANOG and 78% LIN28) (Supplementary Fig. 7).

2.2.2. Central Banking

Retrospective assessment of the experience of the Central Bank with supplied lines is summarised in Fig. 4. Of the 47 *Hot Start* cell line batches deposited (Supplementary Figs. 8–10), 9 failed QC at the Central Bank, 6 of which were able to be recovered from cell stocks and replacement batches produced either by the Central Bank or by depositors directly. Approximately 87% of cell lines recovered well post-thaw, growing to confluence within a suitable timeframe and exhibiting acceptable or very acceptable morphology with low to medium levels of spontaneous differentiation, typical of that of human pluripotent stem cells. Cultivation of the poorly recovered lines necessitated common operator practises of low split ratios at passage and mechanical removal of differentiating cells to adapt lines to implemented protocols and their expansion for quality control at the Central Facility. By the time of cryopreservation all expanded lines were viable and showed acceptable phenotype and pluripotent potential, allowing release and distribution.

All EBiSC banked lines were tested for sterility, mycoplasma and contamination with human viral pathogens (HIV-1 and -2, Hepatitis B & C), the latter by PCR nucleic acid testing. Three of the lines (6%) had to be discarded due to microbial contamination and all but one line

Post-thaw recovery (n=47)	**Recovered well**	**Issues recovering**	**Could not recover**
	87.2%	12.8%	0%
IPSC Morphology (n=47)	**Very acceptable**	**Acceptable**	**Unacceptable**
	55.3%	29.8%	14.9%
Spontaneous Differentiation Level (n=47)	**Low to Medium**	**High**	
	85.1%	14.9%	
Sterility (n=47)	**Contamination Not Detected**	**Contamination Detected**	
	93.6%	6.4%	
Viral Screening (n=46)	**Viral Pathogens Not Detected**	**Viral Pathogen Detected**	
	98%	2%	
Mycoplasma (n=45)	**Mycoplasma Not Detected**	**Mycoplasma Detected**	
	100%	0%	
Cell Line Identity (n=47)	**Correct**	**Incorrect**	
	83.0%	17.0%	
Pluripotent Potential (n=38)	**Trilineage Differentiation**	**Incapable of forming 1 or more germ layers**	
	100%	0%	

Fig. 4. Quality Control at Central Bank. Release of cell lines for sale and distribution was dependent on passing core release, quality control screening performed at the Central Bank whose outcomes for each parameter is assessed in relation to the total number of lines committed by supplying centres ($n = 47$).

(98%) was negative for HIV-1, HIV-2, HBV and HCV. This line resulted as positive for HIV-1 but further investigation discovered a cell line identity error and the depositor was unable to identify the correct source material, the most frequent of QC fails identified. Using PCR to assess DNA microsatellite markers, the identity of 17% of deposited cell lines differed from that intended. Potential root causes for these were not fully confirmed but were likely to include switching of cell lines at cryopreservation, concurrent cell processing of different lines or mislabelling during reprogramming and processing.

2.3. The Hot Start Collection

Based on information registered on hPSCreg for the 27 *Hot Start* lines available for distribution at the time of the Catalogue launch, we compared the collection against the total population of hiPSC lines on the registry for which information has been validated. At time of launch this amounted to 283 hiPSC lines from 17 iPSC supplying centres, excluding the available 27 *Hot Start* lines. Results are depicted as a ring graph in Fig. 5, with corresponding numerical and percentage values tabulated in Supplementary Fig. 11 A, B). Diseases represented by available lines were broadly classifiable as Neurological Disorders (37%), Cardiac Disease (19%), and Eye Disease (15%). Approximately 30% of the *Hot Start* lines were sourced from disease unaffected donors, as compared with 72% on the registry. For both the collection and lines on the registry, the majority (78–85%) originated from fibroblast cells. Blood erythroblasts were also included in both. Over two thirds (74%) of the *Hot Start* collection were created using a range of non-integrating vectors, namely RNA (Sendai, 37%), episomal plasmid DNA (26%) or excisable transposon (11%) vectors. A comparable proportion of hiPSC lines on hPSCreg were created using non-integrating methods, predominantly Sendai virus (70%). The overwhelming majority of all lines in the *Hot Start* collection (81%) and hPSC registry (86%) were reprogrammed using the original Oct4 (POU5F1), Sox2, KLF4, c-Myc combination as originally reported by Takahashi et al., (2007). As regards culture systems used originally to derive the lines, 74% and 26% of the *Hot Start* lines were derived in mTESR and E8, respectively as compared with 4% and 3% for lines on the registry. The predominant matrices were Vitronectin (53%, registry) and Matrigel/Geltrex (74%, *Hot Start*). In both cases the predominant method of cell passaging was EDTA (100% versus 56%, *Hot Start* vs registry). We intentionally sought gender balance in the *Hot Start* collection based on availability at time of banking (52% vs 48% m:f).

3. Discussion

The EBiSC *Hot Start* yielded a distributable resource of 27 pre-existing hiPSC lines constituting lines for which depositors had the greatest available data and working experience. As compared with EU registered lines, hiPSC in the *Hot Start* Collection also predominantly originated by reprogramming of fibroblasts by the canonical OKSM combination of transcription factors. However, the proportion of lines reflecting use of more modern technology and methods for hiPSC line derivation was greater in the collection than for lines on the registry across the spectrum of other modifiable parameters (i.e. media, matrices, passaging method, reprogramming vectors).

Key lessons arising from the Hot Start experience included the following:

1. *Depositor priorities*. Although facilitating third party user access to hiPSC lines is central to the EBiSC mission the first bottleneck to

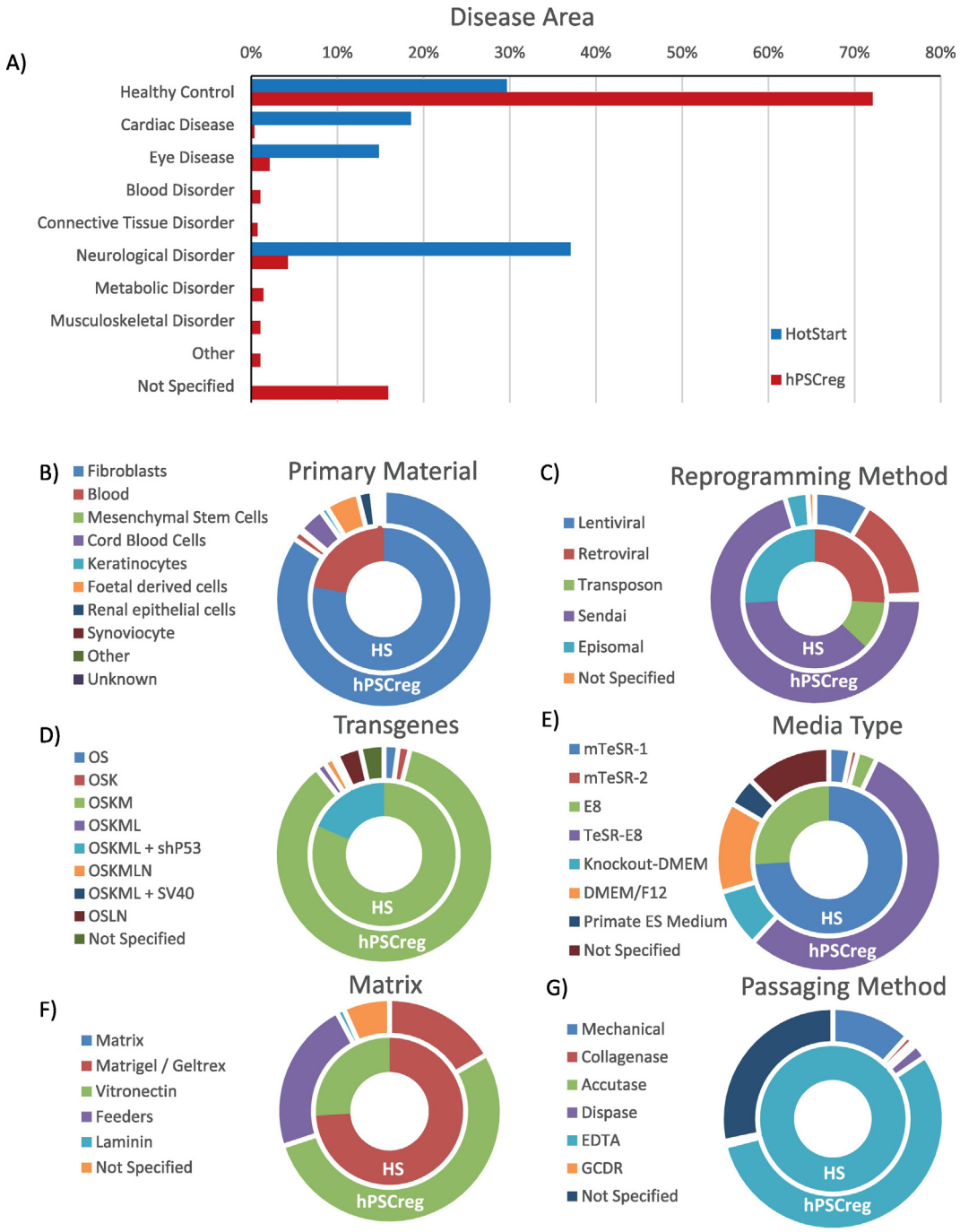

Fig. 5. EBiSC *Hot Start* collection representativeness in relation to hiPSC validated in hPSCreg. A) Disease association of hiPSC donors from the *Hot Start* collection (blue) compared to publically viewable hiPSC lines in the hPSCreg database; Additional cell line characteristics compare hPSCreg data (outer circle) versus the *Hot Start* collection (HS, inner circle): B) primary material for reprogramming; C) reprogramming method; D) transgenes used in reprogramming; E) culture medium; F) culture matrix; G) passaging.

such access rests with the depositor. Reluctance to make a line available to the wider research community and competitors prior to publication or commercial development may slow the pace with which the benefits offered by banking initiatives are realised.

2. *Data and stock storage*. Delays due to issues obtaining and collating historical cell line data were common and retarded progress. Additionally, multiple issues were observed around poor prior labelling of cryopreserved cell line stocks, leading to both cell line identity failures and irreplaceable loss of cell batches, underscoring the importance of cell line identity checking by microsatellite DNA assessment.
3. *Legal governance*. The lengthy negotiations involved in the initial formulation of EBiSC template agreements pertaining to material deposition and distribution, and the institution-specific adaptations required to facilitate their implementation, ultimately impeded the rate at which cell lines were deposited and made available for distribution. The obstacle presented by Spanish law, which precludes the distribution of Spanish donor derived pluripotent stem cells (hiPSC and hESC) by an entity other than a Spanish authority, required testing and the boundaries set by the EBiSC project provided the perfect setting for engagement to explore what future adjustments to legal constitution might be feasible. Other legal or cultural norms, such as a protective patient-clinician relationship, data protection laws, or requirement of absolute donor anonymity may be found to impose further restrictions on efforts to globalise procurement.

4. *Robustness of available protocols and hiPSC resources*. Despite the centre wide diversity of hiPSC line provenance (i.e. cell of origin, reprogramming, culture and cryopreservation methods) the programme was successful in implementing standardised feeder-free culture conditions (Ludwig et al., 2006; Chen et al., 2011) using commercially sourced reagents. The provision of several training courses was perceived as critical for this. The robustness of these feeder-free protocols was further substantiated by a pilot study undertaken in collaboration with another IMI funded initiative, StemBANCC (www.stembancc.org) focused on provision and use of well characterised patient derived hiPSC for use in drug discovery, wherein lines banked under feeder-dependent cultures were transitioned directly and banked under feeder-free conditions (data not shown).
5. *Value of batch testing for sterility and identity*. The importance of measures to safeguard against microbiological contamination and mislabelling of cell lines is broadly understood and avoidable through implementation of high quality standards of operation (Stacey et al., 2013). The danger of procuring lines from sources other than banks routinely implementing these measures as a batch release criterion is underscored by our experience. The incidence of mycoplasma contamination is estimated to be around 5–30% of cell lines across the world (Nikfarjam and Farzaneh, 2012). Although the number of cell lines screened was modest (i.e. 47) and centres sampled was small ($n = 7$), mycoplasma were not detected in any deposited hiPSC line as assessed by both direct colony growth and a high sensitivity, indirect qPCR based method. Checking for culture sterility by inoculating liquid microbiological growth media with spent culture media and incubation for 14 days, rather than less stringent visual assessment of cell cultures in process, revealed only a low incidence (3/47) of bacterial contamination of undetermined type. This was thought to arise from the occurrence of low level bacterial contamination which was not observed, even in antibiotic-free cultures due to the daily media changes applied to cultures but may also have been due to variability in contamination levels in the laboratory of origin.
6. *Labelling*. Defining traceable nomenclature and labelling terms involved substantial and iterative inter-institutional discourse. Initially, the local cell line name was added as a safety measure to ensure the correct labels were used during cryopreservation. The switch to 2D barcodes was made to improve barcode scanning compatibility with an established automated cryostorage system. Due to repetitive cell line identity failures and/or incorrect clone IDs recorded prior to deposit, the local cell line name was subsequently removed. Co-incidentally the distributing centre (ECACC) catalogue number was introduced.
7. *Interface with established public resources managing information and cell lines*. Key to the future sustainability and quality of EBiSC resources initiated by the *Hot Start* process is the interface and integration of well established public resources for data and cell management. For example, the hPSCreg project is independently funded by the European Commission to provide a central resource of hPSC lines along with ethical provenance, and it has emerged as the established community standard for iPSC registration (Seltmann et al., 2016). hPSCreg issues certificates to verify that a registered line meets strict minimal data requirements, and this certificate is now required by a cell line before it can be included in proposals for EC research funds. It also provides the portal for registration of lines for banking and distribution by EBiSC. A fast track registration process for EBiSC lines has been set up to enable rapid processing for accession of EBiSC lines although certification of lines will still require completion of hPSCreg minimal requirements. A guide for registration has been prepared to assist depositors (http://www.ebisc.org/files/Other-doc/01-QuickStartGuideCellLineRegistration-2016-06-14-V2.pdf). EBiSC automatically registers all of its hiPSC cell lines in the BioSamples database at EMBL-EBI. This ensures that any hiPSC assay data, generated on EBiSC cell lines and deposited in the EMBL-EBI archives (such as RNA-seq or array data) can be directly linked to the EBiSC catalogue enabling rapid user access to molecular data where it is available. EBiSC uses ontology tools hosted by EMBL-EBI to annotate cell lines using a controlled vocabulary, which ensures cell line scientific data are accurately described and more easily discoverable in the web portal. EBiSC has arranged for cell line data to be shared, seamlessly and automatically, between the different components of the IMS network ensuring that the data is up to date and content is validated when released to the public. This includes hPSCreg, the IMS database, ECACC, the central facility's Laboratory Information Management System (LIMS), and the Biosamples database. Depositors and prospective users/customers benefit from the assurance that cell line data is consistent across these services, for all cell lines. Lastly the IMS provides a user-friendly application layer for searching across hiPSC lines and associated datasets within the EBiSC collection and provides support to internal business operations and management of cell line data.
8. *Donor information*. At present hiPSC donor information supplied and publicly available through such capacity as provided by hPSCreg is limited. There is broad recognition in the field of worries in gathering and making detailed clinical information and medical records publicly available in relation to privacy and consent associated issues (Peppercorn et al., 2012; Bastião Silva et al., 2015). From a 'database point of view' the presentation of clinical data as free text is not preferred as this complicates searching and comparing clinical information between donors. Rather, standardised taxonomies and ontologies should be developed and used to describe a disease and there are ongoing efforts to this effect amongst pertinent institutions within EBiSC (EBI). Existing standards such as Systematised Nomenclature of Medicine (snowmed), the International Classification of Diseases (ISO), or disease ontologies should be applied if possible. At the moment, hPSCreg registers clinical data related to diagnosed disease(s) based on experimental factor ontology (EFO) (Malone et al., 2010) and disease ontology (DO) (http://www.disease-ontology.org) standard terms by applying a semantic mapping tool Zooma (Sarntivijai et al., 2016), but also allows for free text information. In addition, genetic disease carrier status, family history, disease- and non-disease related phenotypes can be provided as free text. Furthermore, disease - and phenotype - associated mutations/polymorphisms can be registered for each of the diagnosed diseases of a donor, also using standard nomenclatures of the Human Genome Variation Society (HGVS). The already implemented standardised attributes allow direct comparison and searching, without additional free text analysis. HPSCreg also asks depositors whether medical history files or clinical information for the donor are available upon request. Compliance with providing clinical and genetic donor information together with cell line information is a major bottleneck and could be helped by incentive or assistance to depositors provided by banking establishments such as EBiSC.

In summary, we describe here the feasibility and challenges of coordinating existing organisational capacities across Europe to fast track the establishment of a centralised network and facilities to access a standardised resource of established hiPSC lines and data. The availability of these should benefit the accessibility, comparability, reproducibility and efficacy of ongoing research on this resource in all sectors, public and private. Additionally, EBiSC experience and infrastructure can benefit future efforts to standardise the procurement and derivation of new hiPSC lines. A prospective depositor's early engagement with EBiSC could aid in the avoidance of costs of local banking and quality control not to mention provides a safe off-site depository. To this end, EFPIA member sponsored production of hiPSC lines, whose costs are partly subsidised by EBISC, are ongoing for diseases for which there is researcher demand not currently met by existing supply.

Recently, guidelines prepared by the International Society for Stem Cell Research (ISSCR) for global standards for stem cell research and

clinical translation in 2006 and updated in 2008 were updated further (see Commentary, Daley et al., 2016). Review of these to confirm complicity and alignment of hiPSC lines banked as part of EBiSC *Hot Start* launch verified that for the guidelines which were applicable (eg. procurement, processing and banking) EBiSC hiPSC lines either complied or enable compliance by third party users (Supplementary Fig. 12). This makes the EBiSC *Hot Start* collection and ongoing advances in infrastructure of broader global benefit to other small and large scale hiPSC derivation and banking initiatives.

4. Experimental Procedures

4.1. hiPSC processing

Protocols used for feeder-free hiPSC culture, passaging and cryopreservation (available on line: http://www.ebisc.org/files/Other-doc/EBiSC-User_Protocol-V2-2015-06-11-V0.1.pdf) and consisted of two methods consisting of mTESR and Matrigel/Geltrex or E8/Vitronectin culture in 5% CO_2 in air (37 °C), with EDTA based cell passaging (Ludwig et al., 2006; International Stem Cell Initiative Consortium, 2010; Chen et al., 2011). At time of cell harvesting each batch of cells were lifted from individual vessels and pooled prior to dispensing into pre-labelled sequentially numbered vials. Lines were cryopreserved as aliquots of 1–2 × 10^6 cells per vial as clumps of cells in either Cryostor or DMSO based freeze mix of 10% DMSO in FBS and/or culture medium.

4.2. Identity testing

Identity testing was performed by 16 allele STR profiling (PromegaPowerplex 16, performed by Source Biosciences).

4.3. Viability testing

Viability testing was performed by observation of culture recovery over 48 h and assessment of cultures graded according to number and appearance of typical hPSC colony morphology. These were subjectively assessed as very acceptable, acceptable or unacceptable.

4.4. Mycoplasma testing

This was performed by RT PCR using appropriate limit of detection controls (MycoSeq, Life Technologies). A standard 28 day culture screening was also performed (ECACC and mycoplasma experience).

4.5. Sterility testing

Sterility testing was performed according to methods based on the European Pharmacopeia standard. Specifically, 0.5 ml to 1 ml of cell culture supernatant were aseptically inoculated into sterile Tryptone Soya Broth (TSB), Fluid Thioglycollate Medium (FTM) and Sabaroud Dextrose Broth (also known as Sabaroud Liquid Medium, SLM, where performed at NIBSC) respectively and incubated at 37–39 °C (THB, FTM) or 20–25 °C (SLM) for up to 14 days and observed periodically (on days 3, 4 or 5; 8, 9 or 10) and on day 14 for evidence of microbial growth.

4.6. Flow Cytometry

The proportion of the population expressing stem cell markers, SSEA-4, SSEA-1 and TRA-1-60, was assessed (BD Biosciences) on the BD Canto II flow cytometer according to manufactures instructions.

4.7. Pluripotency assay

Cell lines were assessed for differentiation potential using embryoid bodies (EBs) and germ layer gene expression assessed using quantitative PCR. Media was removed and the cells were washed with PBS. TrypLE™ was added for 3 min. DMEM containing 20% FCS was added to quench the TrypLE. The cells were centrifuged at 300*g* for 3 min and then resuspended in Apel™ media (Stem Cell Technologies) containing 10 μM Rock inhibitor at 30,000 cells per ml. 100 μl was then added to each well of a U-bottom 96 well plate and centrifuged at 300*g* for 3 min. The plates were place in an incubator at 37 °C, 5% CO_2. EBs formed overnight and samples were collected at days 7 and 14. RNA was extracted using Maxwell RSC machine and kit, according to manufactures instructions (Promega). EB-derived cDNA was produced using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to manufacturer's instructions and analysed with gene-specific probes (Applied Biosystems) by standard methods and run on Quantstudio thermocycler (Thermofisher). For assessment of cell line pluripotency each differentiated sample was compared against its undifferentiated counterpart as a normalised control using the DDCt method, to give relative quantitation (RQ) values using GAPDH and ACTB as reference genes. Each batch of cells used to create EBs were tested using specific antibodies to SSEA-1, SSEA-3, SSEA-4 and TRA1–60 (R&D Systems) on the BD Accuri flow cytometer according to manufacturers' instructions.

4.8. Validation of hiPSC phenotype by high content image analysis

hiPSCs were seeded at a density of 1000–4000 cells/well on 96-well μClear® cell culture plates (Greiner Bio One) pre-coated with Matrigel. Adherent cells were washed with PBS and fixed in 4% paraformaldehyde in PBS for 20 min, then washed three times with PBS. Cells were then permeabilized with 0.1% Triton X-100 for 10 min at room temperature and washed 3 times with PBS. Cells were then blocked with serum (3–5%) from the antibody producing host species in PBS for 30 min. Incubation with the respective primary antibodies in blocking solution was performed overnight at 4 °C (Oct-3/4, sc-8629 (goat), Santa Cruz Biotechnology Inc., 1:500; Sox2, sc-17320 (Y-17) (goat), Santa Cruz Biotechnology Inc., 1:100; Lin28, 11724-1-AP (rabbit), Proteintech, 1:300; Nanog, ab109250 (rabbit), abcam, 1:500). On the following day the cells are washed 3 times with PBS. Alexa Fluor 488 (optimal for Opera High Content Imaging System laser configuration) secondary antibodies were diluted 1:500 in blocking solution and incubated with the cells for 4 h at room temperature in the dark. Cells were then washed 3 times with PBS. Nuclear counterstaining was carried out with 2 μM Hoechst 33258 (Invitrogen). Cells were analysed on an Opera High Content Imaging System in combination with the Columbus Image Data Storage and Analysis System (PerkinElmer). Laser power and exposure times were adjusted to linear detection range to avoid detector saturation. Automated imaging was performed using 20× objectives and a sub layout with 45 image fields per well (covering representative and defined parts of the wells), resulting in image acquisition rates of approx. 1500 cells per well. For image analysis readily useable building blocks implemented in the Columbus software (software version 2.6, PerkinElmer) were applied. The image analysis algorithm sequence was generated with Columbus building blocks ("nuclei detection", "number of objects" (nucleus and cytoplasm), "mean intensity nucleus", "mean intensity cytoplasm", "mean intensity cell", "number of analysed fields").

4.9. Database mining

The hPSCreg database includes data from EBiSC hiPSC – lines as well as non-EBiSC lines. Lines can be registered freely, but public release of lines requires a validation process. Validation is based on the provision of mandatory data, including compliance with standard ethical requirements, data providing sufficient evidence of pluripotency and others related to derivation, cultivation, donor characteristics and data access policy (https://hPSCreg.eu/docs/downloads/QuickStartGuideCellLineRegistration.pdf). We analysed two different categories of hiPSC – lines in the hPSCreg database: (a) hiPSC lines that are registered and validated (released) ($n = 283$ and (b) the *Hot Start*-lines ($n = 27$),

representing a subcategory of (a). Cell line specific hPSCreg-data of each category was pre-processed and normalised with Java. The charts in (Fig. 3) were generated in Microsoft Excel. Human embryonic stem cell lines (hESC), which are also registered in hPSCreg, were not included in the analysis.

Contributions of authors

TA, AC led assembly of consortium, funding application, executive project management.

PDS wrote paper, led work package establishing *Hot Start* hiPSC collection and operational Central Facility. RS, EW, KB, JK, MH, SK, GM, JH undertook operational execution of *Hot Start*, notably collation and review of donor informed consent (KB, SK, MH), liason with supplying centres (JK), labelling (KB, EW), cell line receipt, processing and testing (RS, EW), team management (KB, GM, JH). AK, SS lead management of *Hot Start* hiPSC line data.

JD, SR analysed *Hot Start* hiPSC data. BH, MB, JD, RP Development of EBiSC Information management system and the cells.ebisc.org web catalogue. GS lead on specification of hiPSC Quality Control measures and development of partner training for *Hot Start* laboratory staff. OO'S, CC, LH provision of QC testing (sterility & pluripotency assays), flow cytometry and delivering practical training for *Hot Start* laboratory staff. BB leads on work package for hiPSC storage and distribution and management of incorporation into existing ECACC business model. TR – IT delivery and manager of integration of EBiSC *Hot Start* collection and information management system with ECACC. IA – ECACC's website and marketing manager responsible for website scientific content as well as end user experience. IS, LC, HP, PWH, AF, LC, TB – Data management at EBiSC Central Facility. HZ leads on hiPSC mirror banking. AV, MS, BK, CT, TS, JH. OB, MP, NG, BH, CC, MLP, CL, ML, LA lead and delivery of hiPSC supply centre contributions. TA, SG, AK, RH, AJ, GB, AE, AC-S, PF, MG, TCS: specification of EFPIA requirements, validation of distributed hiPSC pipeline and quality control. OP, OK, PG, CC validation of ECACC distributed EBiSC *Hot Start* hiPSC using high content imaging. CG, SH, hiPSC legal governance. AR, BK, supported consortium building, recruitment of hiPSC centres, funding application, daily project management, design and provisions of communication and collaboration infrastructure and materials. PDS, GS, RS, EW, JK, AK, JD, SR contributed figures and associated text. All authors contributed review and revisions.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2017.03.002.

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